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RESISTANCE AND IMMUNITY IN PLANT DISEASES

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IMPORTANCE OF DISEASE RESISTANCE IN PLANTS

Among the numerous problems of the Plant Pathologist, few occupy such an important position either from the view point of scientific interest or of economic importance as those of resistance and immunity to disease. Immunity offers a safe and most desirable method of disease prevention—unquestionably the ideal method in all cases, where other varietal characteristics of importance are not incompatible with such immunity. Resistance and immunity offer, moreover, in many cases the only apparent feasible remedy for plant disease. When sprays and steeps, rotation and other common means of combating disease all fail, resistance or immunity seem to offer the only hope of success. The rusts of cereals are illustrative of such a condition and the resistance exhibited by certain varieties of durum wheats is indicative of the possibility of the development of resistance in other varieties of small grains.

Not only are many diseases not amenable to any known treatment, but many others can be controlled only at a prohibitive cost. Although combative measures of practical importance may yet be devised for many of these, some will unquestionably resist for a long time any attempts at economical prevention by ordinary mechanical or cultural methods.

Another class of diseases of plants offers the difficulty of host plant injuries due to treatment. Present methods may cause burning of the foliage or they may in other ways disturb the physiologic activities of the host to such an extent as to make them of doubtful use. It is especially in these classes of plant diseases where mechanical or cultural treatments have failed of their purpose, that the possibilities of pre-

[1] Read before the Botanical Society of America, Minneapolis, Dec. 29, 1910. This was the last of three papers in a Symposium on Some Aspects of Plant Pathology. The other two have been published in preceding numbers of this journal.

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vention by the introduction of resistance or immunity offer a field of investigation not only alluring in its scientific aspect, but also reasonably promising of results. The lure of resistance and immunity in disease prevention is especially forceful in the economic saving and ease of application. When resistance or immunity fight the battle against disease, no formula for sprays or steeps so easily misunderstood or misapplied, and no special cultural methods are required. Prevention is purchased with the seed or stock.

On the other hand from the standpoint of a practical working out of the development of resistant varieties, it must not be forgotten that disease resistance may be only one of many important characters sought in the crop under consideration. In the breeding of any crop the complexity of the problem is increased by each new factor under consideration. When any of these can be removed by artificial means, if only temporarily, the whole problem of improvement may be lightened. In view of this fact it may in practice often be deemed inadvisable to pursue the search for resistance even where indications of resistant qualities are clearly shown—at least until the more important demands of the breeder are satisfied. In illustration the following may be cited. It is not generally recognized that there is any considerable resistance in oat varieties to the common smut (*Ustilago avenae*). Experiments at the Minnesota Agricultural Experiment Station (in cooperation with the U. S. Department of Agriculture, Office of Cerealist) have shown that a variety of Burt oats, developed by Norton of the U. S. Department of Agriculture, when inoculated with viable smut spores shows almost no smut, while Early Champion oats under exactly similar conditions produces an abundance of smut. In the breeding of oats many other factors are necessarily taken into consideration, *e.g.* weight of chaff, earliness of ripening, yield, lodging, etc. At a very low cost of treatment of the oat seed the smut may be absolutely prevented. In view of the complexity of the entire problem of oat breeding and in view of the ease and cheapness of the eradication of the smut factor, it would seem that for the present at least the pursuit of smut resistance should assume a position of only minor importance. The question of resistance and immunity must in every case be given its true value in any scheme of selection or breeding. A somewhat similar question arises in the rusts of cereals. Resistance to stem rust (*Puccinia graminis*) of wheat is a far more important question in the spring wheat districts than in the winter wheat states and even in the former, the rust cannot be considered the factor of first importance in breeding when compared with yield and milling and bread-making qualities of the wheat. The absence of

rust resistance in spring wheats results in severe losses periodically but not every year. The farmers who raised resistant durum wheats following the rust epidemic of 1904 are not all now raising durum wheat, because the other factors of milling qualities, market value, etc. have in years when little or no rust appeared, placed the rust question in the proper light of being only one of several important factors in wheat production.

POPULAR MISCONCEPTIONS OF RESISTANCE

If disease resistance is to occupy the place in the work of the plant pathologist which it ought to occupy, it becomes of utmost importance that popular misconceptions regarding the nature of disease resistance be corrected, in order that the work may not be hampered by unearned criticism and loose terminology. There are many popular misconceptions of disease resistance. Prominent among them are on the one hand the supposed ease of production, and on the other hand those arising from a confusion of terms and ideas. In the popular mind and even in a good deal of scientific literature any variety that escapes a particular disease is classed as a resistant variety. Orton has clearly pointed out the differences between disease-escaping, disease-enduring, disease-resisting and immune varieties. There is little difference in the real resistance powers of oat varieties to the common oat rusts, and when a grower is told that an oat variety is resistant because it usually escapes the rust through earliness of ripening, he is led into a serious mistake which will sooner or later throw discredit on the work of the pathologist. The essential character of true resistance as pointed out by Ward[1], Salmon[2], Biffen[3], Orton[4] and others lies in a protoplasmic activity and is independent of inoculation accidents. A variety may escape a disease through certain peculiarities of physiologic host activities, of meteorological conditions, or even of certain morphological host characters. The latter is well illustrated by the following experiments of the writer. A variety of barley was grown in different soils provided by Harter of the U. S. Department of Agriculture during his alkali soil investigations. These soils varied from the normal garden soil to those containing about 2% of alkaline salts. Plants in the different soils when inoculated by spraying methods in the greenhouse showed different amounts of rust, in general those in the stronger alkaline soils showing less rust. The latter, however, when attacked exhibited large and vigorous growths of the rust. The smaller percentage

[1] Proc. Roy. Soc. **47**: 441 (1890).
[2] The New Phytologist, Vol. IV, No. 9, Nov. 1905.
[3] Journal Agr. Science, **1**: 4-48 and Journal Agr. Sci. **2**: 109-128.
[4] Yearbook of U. S. Dept. of Agr. for 1908.

of pustules was undoubtedly due to the greater development of "bloom" on the barley foliage when grown in the strong alkali, resulting in the rolling off of the water drops, and hence in the loss of the inoculating material. Harter[1] has shown that the plants in the different soils exhibited measurable differences in the thickness of the outer epidermal walls. The bluish color of the plants in the alkaline soils and the greater tendency of the water drops to run off were quite pronounced. There seems to be no reason for assuming that there was any difference in real rust resistance. Again Ward has shown that wheat rust uredo spores may send germ tubes into the stomata of plants of many different genera other than barberry, wheat or other grasses, and that they may even produce a rudimentary mycelium but they fail to penetrate the protoplasmic defense of the mesophyl and soon succumb.

The economic importance of disease-enduring varieties ought not to be overlooked by working pathologists. It seems probable that exceedingly effective work in selection and breeding may be accomplished in the production of disease-enduring varieties. The best varieties of the hard spring wheats commonly used in the north central states, both of blue stems and fifes come under this class in regard to stem rust, since not one of them possesses any appreciable amount of resistance. They can endure light or even moderate attacks of rust, but all go down quite uniformly under uniform conditions in a heavy epidemic. It is of course obvious that a sharp line of demarcation may not exist between disease-enduring and disease-resistant varieties. For practical purposes of selection and breeding, however, well marked resistance can readily be detected under proper experimental conditions.

Immune varieties possess perfect resistance. Their production will probably always remain rare in comparison with those possessing only partial resistance or high powers of endurance. In this discussion the term "resistance" is used only in reference to true resistance.

PARASITISM AND RESISTANCE

Although the literature on parasitism in its various phases is voluminous, a great deal remains to be done in clearing up important problems. That there are widely different types of parasitism cannot be doubted by any observer who studies closely such contrasting parasitic forms as *Pythium* and *Puccinia*. DeBary long ago recognized differences in parasitism and modern research has assisted in still further clearing up questions of detail in parasitic problems. Between the parasitism which results in immediate death of the host

[1] U. S. Dept. Agr., Bur. Pl. Ind., Bull. 134.

plant tissues attacked, and the finely adjusted parasitism of a smut or rust, where a condition hardly distinguishable from symbiosis occurs, there is a wide range. The fungus of *Lolium temulentum* offers an example of parasitism which is quite generally admitted by those who have worked with this interesting fungus to be essentially symbiotic. There seems to be no reasonable doubt that this fungus is a smut adapted to intra seminal infection of the embryo. Lichenologists exhibit of late a tendency to emphasize the parasitic nature of the fungus symbiont, leading naturally to a re-arrangement of the lichen classification. Of course there are many widely varying views as to the delimitation of the term "symbiosis." However close or loose the association of the symbionts, a beneficial inter reaction is generally presupposed. There may be a considerable difference, however, in the interpretation of phenomena as to whether the beneficial action is to be required for the symbionts as a whole or for only restricted areas of the symbionts. For instance, green islands such as are sometimes found in powdery mildews and also in cereals rusts, seem clearly to indicate that in comparison with the extra-mycelial host tissues, those tissues immediately attacked by the mycelium are preserved in a green living condition for a longer time. Judging only from the effects at the point of attack, such conditions might well be called symbiotic. The ultimate effect on the entire host is, however, far from beneficial. It seems clear therefore that the common conception of parasitism includes many different conditions. The exact nature of the cytologic and physiologic forces which make possible or impossible a parasitic reaction are only partially understood and have been studied only in a few forms. Ward has shown that the resistance offered to the invasion of a rust mycelium in grasses such as *Bromus* resides in the protoplasm of the mesophyl cells, but the exact forces of resistance were not determined. The possible role of enzymes in such inter-reactions is suggestive and the recent activity in the study of enzymes from the standpoint of both the biologist and chemist bids fair to throw much needed light on cell activities.

INHERITANCE OF DISEASE RESISTANCE

In the absence of more detailed information in regard to the cytologic or physiologic forces operative in parasitism and resistance, little can be said of the inheritance or resistance to disease. Biffen has carried on extensive experiments with rust (chiefly *Puccinia glumarum*) in wheats. Rust resistance according to his results can be regarded as a unit character behaving in crosses of resistant and susceptible varieties as a Mendelian unit character. The resistance

to rust was found to be recessive. These results can be applied of course only for this particular class of parasitism. That other classes would behave similarly does not necessarily follow. The collection of experimental data in regard to the inheritance of resistance in breeding is of the highest importance.

THE PRODUCTION OF RESISTANT PLANTS

The production of disease resistant plants is of the utmost importance to the plant pathologist as a means toward disease prevention. Disease-escaping and disease-enduring forms may on many occasions also prove useful. The possibilities of selection, variation, mutation, etc., are similar to those in any line of plant breeding. As an accessory of the greatest importance the experimental field or disease plot deserves particular emphasis. In all lines of selection and breeding for resistance control of the field conditions is necessary. The investigator must be able to obtain with reasonable certainty an epidemic when it is desired. Such disease plots must be under a control equivalent to the accuracy of laboratory control if reliable results in the fundamental problems of disease resistance are to be obtained. Such a control is sometimes possible even under very adverse conditions. The following will illustrate. At the Minnesota Agricultural Experiment Station cereal breeding investigations for rust resistance have been carried on for several years. The season of 1909 was an unusually dry one and the rust in the grain fields at the Station was negligible in amount. By artificial inoculation and control a very severe epidemic of stem rust was obtained in the rust plot (1½ acre), while fifty feet from the plot the fields were clean of rust. The season of 1910 again was even drier than that of 1909, the rainfall for the year being the lowest ever recorded in the state and amounting to about one-third of the average total, and the growing season was exceptionally dry. Yet even under these conditions a fair rust epidemic was obtained in the rust plot (1 acre), while in neighboring fields hardly a trace of rust could be found. The experience gained by minor mistakes in the control of this year would probably enable us to obtain in another year of this sort a very heavy epidemic. Artificially controlled disease plots make possible the working out of numerous field problems with a greater degree of accuracy than can be obtained in ordinary field observations. The value of the control of the disease attack is especially great in selection. In some cases a gradually increasing severity of the disease may be sought for, while in others a very severe attack may be desired from the first. In the development of disease-enduring varieties the disease plot is indispensable, where a gradually in-

creasing severity of disease attack makes possible the gradual elimination of undesirable forms.

It seems highly probable that much valuable work in the development of disease resistance will result from hybridization. Biffen's work has already shown the possibilities. Very often it happens that those varieties or species possessing true resistance are worthless from other view points, whereas no variations from the susceptible but otherwise desirable varieties possess in the slightest degree such resistance. Under these conditions crossing is the only apparent means of combining such characteristics.

Although disease resistance offers the most nearly ideal solution of the problem of disease prevention, two difficulties must be borne in mind. Disease resistance may comprise resistance only to one particular disease and a variety as *e. g.* of flax which may be highly resistant to wilt, may on the other hand be highly susceptible to rust. Combinations of resistance and endurance may have to be sought under such conditions. In the second place the possible variations or mutations of the disease organism must be constantly kept in mind. An established or at least apparent resistance may be completely upset by such developments in the parasite. Resistance therefore must not only be established, but must also be maintained.

IMPORTANT PROBLEMS

Among the many problems for future solution with which the plant pathologist is confronted, the most important of those dealing with the resistance of plants toward disease may be briefly enumerated: Experiments, extensive in scope and established in many different localities, on the nature of parasitism of different plant disease types in order to more clearly understand the conditions of parasitism; a study of the role of enzymes in the cytological forces and the possibilities of their use in the cell defense; the effect of physiological conditions on powers of resistance or susceptibility and the possibilities of disease resistance through a more perfect control of such conditions, *e. g.* work analagous to the chemotherapy of Ehrlich in the investigation of human diseases; the further investigation of many types of resistance as to their conformity to Mendelian laws; and the possibilities of mutations.

Not only is the field inviting to the professional pathologist but it offers many interesting problems to the botanist who may not necessarily be concerned in the economic phases of the results.

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THE ASCOGENOUS FORM OF THE FUNGUS CAUSING DEAD-ARM OF THE GRAPE[1]

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We have had under investigation since 1906, the disease of the grape commonly called "dead-arm" by growers. This is the disease which Dr. Reddick has described as "Necrosis" of the grape. Necrosis is an appropriate name but unfortunately it had already been applied by Cavara,[2] as indicated by Sturgis,[3] to another disease of the grape attributed to *Bacillus vitivorus* Bacc. The fungus causing dead-arm was described by Reddick[4] as a new species, *Fusicoccum viticolum* Red.

On account of our present scanty knowledge of a number of the imperfect fungi described by Berkeley and Curtis, and other early American mycologists as occurring on the grape, it is quite possible that the fungus had already been named. This question, however, must remain unsettled until the type specimens of some of these species can be studied.

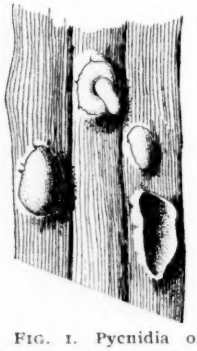

FIG. 1. Pycnidia of *Cryptosporella viticolum* on dead grape vine. The upper one shows a spore mass extruding from the ostiole.

Having tried by using pycnospores of the fungus from different sources at different times and by varying the conditions and culture media to produce the ascospore stage in pure culture without success, our attention was turned to the study of the different ascogenous forms sometimes found more or less closely associated with the pycnidia on dead grape vines. Ascospores of a species of Diaporthe were first tried. These produced a somewhat similar pycnidial form but clearly different from the dead-arm fungus. We finally found a Pyrenomycete, apparently a species of Cryptosporella, on a grape shoot evidently killed by the dead-arm fungus, which had closely associated with it pycnidia of *Fusicoccum viticolum*. Plates were poured, using ascospores from this material and single ascospores carefully isolated and marked. After germination they were transferred to tubes of corn meal agar.

[1] Read at the Second Annual Meeting of the American Phytopathological Society, Minneapolis, Minn., Dec. 29, 1911.

[2] Cavara, F. Intorno alla Eziologia di Alcune Malattie di Piante Coltivate, Le Stazioni Sperimentali Agrarie Italiane, **30** : 487. 1897.

[3] Sturgis, W. C. Lit. of Plant Disease, Rept. Conn. Agr. Exp. Sta. **1900** : 271.

[4] Reddick, D. Necrosis of the Grape Vine. Cornell Univ. Agr. Exp. Sta., Bull. 263 : 331. 1909.

The ascospores germinated freely in about twelve hours, usually sending out one germ tube from the side or end. The first growth was white with the hyphae spreading in a circular manner. At the end of twelve days a few pycnidia were found in the poured plates. These showed only the ordinary pycnospores and were not certainly known to have been derived from ascospores. At the end of twenty-eight days the single ascospore tube cultures showed a few pycnidia which were found producing both pycnospores and scolecospores. The term "scolecospore"[1] is applied to these long, slender, crooked bodies tentatively to distinguish them from the ordinary pycnospores, because we regard them as reproductive organs, though they have not yet been found to germinate under the ordinary cultural conditions in which the other pycnospores germinate. Reddick regarded them as free paraphyses, but true paraphyses are not normally abstricted and set free as these are, and occur typically only in connection with the asci, though the apparently sterile hyphae sometimes found intermixed with sporophores in pycnidia and acervuli have been so designated by some mycologists. These slender curved spores are evidently identical in character and origin with the so-called stylospores of Diaporthe as described by Nitschke,[2] the other pycnospores being called spermatia by him. In all the single ascospore cultures the pycnidia, with the pycnospores and scolecospores, were practically identical with those produced from pycnospores of *Fusicoccum viticolum* taken from diseased grape vines.

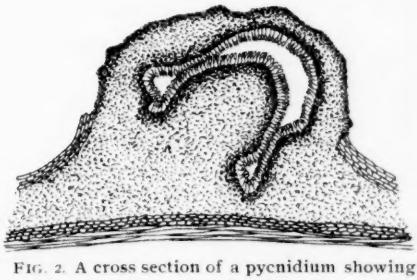

FIG. 2. A cross section of a pycnidium showing a portion of the irregular chamber within which the pycnospores are produced.

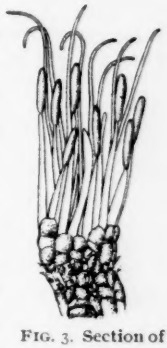

FIG. 3. Section of sporogenous tissue showing ordinary pycnospores still attached to sporophores; also the slender curved spores referred to as scolecospores.

These results appear to leave little doubt that this Cryptosporella is the ascogenous stage of the dead-arm fungus. Though these cultures have been kept growing for about a year, no ascogenous form has appeared.

The same perithecial form has been collected in small quantity on dead grape vines at Lawton, Michigan, and Vienna, Virginia. The material from which these cultures were made came from Clementon, N. J. The perfect stage of the fungus is apparently

[1] From Greek *scolex*, an earth worm.

[2] Nitschke, Th. Pyrenomycetes Germanici. 240. 1870.

rather rare and possibly not a very important factor in the distribution of the disease. The pycnidial form appears to live through the winter and is apparently capable of perpetuating the organism from year to year.

The genus Cryptosporella to which we have tentatively referred this species was described by Saccardo in Michelia, I: 30, June, 1877, as follows (Translation):

"Perithecia valsiform, aggregated, corticular; asci 8-spored without paraphyses; ascospores ovate or fusoid, continuous and hyaline. The genus is distinguished easily from Cryptospora which has vermiculate spores." Ten species, one new, were referred to the genus in the following order.

FIG. 4. Piece of grape vine with perithecial stromata of *Cryptosporella viticola*.

C. hypodermia (Fr.)
C. aurea (Fckl.)
C. Aesculi (Fckl.)
C. populina (Fckl).
C. sphaerostoma (Nke.) sub Valsa
C. compta (Tul.) sub Valsa
C. Daldiniana (Dutrs.) sub Endothia
C. Ehrenbergii (Tul.) sub Valsa
C. foraminula (Pers.) apud Curr. Comp. Sph. n. 131 fig. 127
Sphaeria Limminghii (West.) Prodr. Fl. Bat. p. 47 (in Ulmo) forte eadem est ac *C. hypodermia* (Fr.)

and the new species *Cryptosporella Veneta* Sacc. This was found on branches of *Populus Tremula* in Italy. It is said to differ from *Cryptospora Aesculi* (Fckl.) which has larger spores, less exerted and thicker ostioles.

The dead-arm fungus differs from the above generic description in having perithecia arranged in a more irregular stroma and the asci accompanied by paraphyses. The presence or absence of paraphyses is however of doubtful value for separating genera in this group of fungi.

The type of the genus, *Cryptosporella hypodermia* (Fr.) Sacc. has been grown by Brefeld[1]. He obtained in pure cultures a pycnidial form very similar to *Fusicoccum viticolum* except that no scolecospores were found.

Our fungus shows rather close affinities to Diaporthe in several respects and especially in the production of two forms of pycnospores in the same pycnidium. It differs from that genus chiefly in having

[1] Brefeld, O. Untersuch. aus dem Gesammtgeb. Mykol. Heft 10 Ascomyc. II. 251. 1891.

continuous ascospores. Typical Diaporthe ascospores are said to be two-celled, though it is doubtful whether there is always a true septum present in the spores. Owing to our imperfect knowledge of the life histories of typical species of many related genera it is impossible to refer species with certainly to their proper genus at present.

FIG. 5. Asci and paraphyses of *Cryptosporella viticola*.

If the name applied to the ascogenous stage of ascomycetes is to be regarded as the only valid name of the organism as a whole, the fungus may be called tentatively *Cryptosporella viticola* and characterized as follows:

Cryptosporella viticola N. SP.

I.* Pycnidia very variable in size, structure and arrangement, with irregular labyrinthiform chambers; ostiolate but frequently rupturing and the whole upper half of the pycnidium falling away (Fig. 1.) Hyaline, nonseptate pycnospores of two kinds occur in the same pycnidium; one subfusoid, straight or slightly curved 7.5–15 × 2–5μ; the other scolecospores borne in the same pycnidium, long, slender, variously curved or hooked 18–30 × 1–1.5μ (*Fusicoccum viticolum* Reddick). Figs. 1–3.

II. Perithecia are buried in irregularly pulvinate subcorticular stromata. They are thin walled, globose, furnished with a short, stout, smooth, slightly exserted beak. The asci are sessile or subsessile, 60–72 × 7–8μ. Paraphyses present, slender, septate, wavy, exceeding the asci. Ascospores sub-elliptic, obtuse, hyaline, continuous 11–15 × 4–6μ. Figs. 4–5.

U. S. DEPARTMENT OF AGRICULTURE,
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ANTHRACNOSE OF SCHIZANTHUS

C. N. JENSEN AND V. B STEWART

(WITH ONE FIGURE)

In the fall of 1910, the attention of the writers was called to the diseased condition of the *Schizanthus* plants growing in the greenhouse of the Plant Breeding Department of Cornell University. The disease was observed to be quite disastrous and of sufficient importance to demand some systematic study.

Symptoms. The symptoms of the disease vary markedly with the age and condition of the plants, as well as the parts affected. The young succulent tissue is in all cases more susceptible than the older woody parts. The first indication of the disease is a water soaked appearance of the infected area on the young succulent stems, branches and petioles. This water soaked area extends in all directions and a marked depression occurs due to the collapse of the tissue. This collapse of the tissue continues until there is a falling over of the stem, branch or petiole at the point of the lesion. After this stage has been reached, there occurs a very rapid wilting of the parts beyond the lesion and death soon follows. This is brought about very rapidly, requiring but a few days after the first indications of the disease. The lesion now turns a light brown and soon becomes dotted with the black acervuli of the fungus.

The older infected plants show large canker-like lesions on the stems and main branches, usually at the base of the petioles. However, many instances occur in which these cankered areas cannot be traced definitely to petiole infection. The formation of these cankers is in every way identical with those of the more succulent tissue except they are not so sunken. This is due unquestionably to the more woody condition of the tissue. Occasionally the lesion continues to progress until the stem of the branch has been girdled. Following this we find marked symptoms of the disease manifested in the leaves. They gradually turn yellow and there is a dying from the tip backward. This results from improper nourishment due to the girdling of the stem.

While the disease is usually confined to the stems, branches, and petioles, it is frequently manifested on the leaves by the production of leaf spots from 1 to 2 mm. in diameter and of a light brown color. The spots are not confined to any particular portion. They are usually small and circular except when occuring on the margin of the leaf.

The injury resulting from them is very meagre compared with that resulting from attacks on other parts of the plants.

Etiology. The cause of the disease has been found to be a fungus of the genus *Colletotrichum* and a species apparently new to science. This fungus was first isolated by poured plate dilution cultures of the spores which oozed from the acervuli formed on an old lesion that had girdled the stem of the *Schizanthus* plant at its base. Since the first isolation, it has been obtained repeatedly from cankers on the older, more woody parts, and from the lesions produced on young succulent tissue.

The fungus has been cultivated upon wheat-straw plugs, potato-decoction agar, nutrient agar, and bean plugs. The bean plugs have been found to be of special value in producing the conidial stage, thus yielding spores for inoculation purposes.

Pathological Histology. For a study of the pathological histology, diseased and healthy tissues were fixed in Gilson's fluid, stained with Haidenhain's iron alum-haematoxylin and counterstained with Congo red. Sections were cut 8μ thick.

Sections through a lesion show the mycelium to be scattered profusely through the diseased tissue and up to the very margin. The healthy parts can be distinguished readily from the diseased in that the cells remain turgid with their contents intact. On the other hand, the cells attacked by the organism show a collapsed condition in which no cell contents are visible. There is no dissolution of the middle lamella by the hyphae. They, however, progress from cell to cell by direct passage through the cell wall. The fact that there is a distinct differentiation between healthy and diseased tissue is in accordance with infection experiments which show the fungus to be an active parasite and in no sense living upon tissue already killed far in advance of the hyphae by the secretion of some deleterious agent.

Germination of spores. Spores from pure cultures were sown in drops of sterile water on sterile slides and placed in Petri-dishes for germination studies. Germination in water has at no time exceeded 30 per cent. The germination of spores, production of appressoria, and germination of appressoria have been observed to take place in 16 hours at room temperature. This, however, is exceptional, the maximum generally occurring after about 24 hours. These tests have been corroborated by examination of spores which had been placed in drops of water on the stems of living *Schizanthus* plants, kept under conditions of high humidity.

The behavior of a spore varies materially according to the position in the drop of water. When it lies in contact with the substratum,

an appressorium is formed immediately upon germination. Occasionally, an appressorium may be seen at each end of the spore. When the spore is not in contact with the substratum no appressorium will be formed unless the germ tube later touches it. (Fig. 1). From

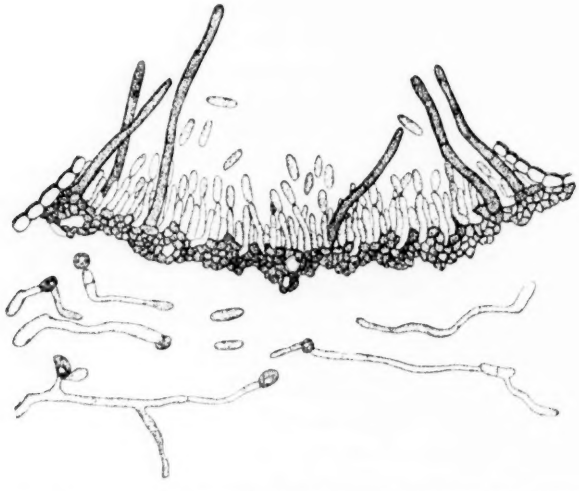

FIG. 1. Acervulus and germinating spores showing appressoria.

this we conclude that the formation of appressoria is due to a contact stimulus as already pointed out by De Bary[1], Büsgen[2], Hasselbring[3] and others.

The spores occasionally become two-celled upon germination. The germ tube originates usually at the ends, seldom at the sides. The appressorium is a thick-walled brown body showing a distinct germ-pore. Germination of the appressorium takes place from the under surface *i.e.* the surface touching the substratum.

Inoculation Experiments. Spores for inoculation purposes were at all times taken from pure cultures. The cultures were removed to water in flasks and shaken up well to obtain liberation of spores from the acervuli. This inoculation water was then transferred to an atomizer and sprayed upon the plants. The water before being used was sterilized in the autoclave for 20 minutes at 125° C, and 17

[1] De Bary, Anton. Ueber einige Sclerotinien und Sclerotininenkrankheiten. Bot. Zeit. **44**:377 et seq. 1886.

[2] Büsgen, M. Uber einige Eigenschaften der Keimlinge parasitischer Pilze. Bot. Zeit. **51**:53-72. Taf. III. 1893.

[3] Hasselbring, H. The Appressoria of Anthracnoses. Bot. Gaz. **42**:135-142. Fig. VII. 1906.

pounds pressure, while the atomizer was first sterilized with 4% formalin for 5 minutes, rinsed in 95% alcohol and rinsed twice in sterilized water.

Inoculations were made between 4 p. m. and 6 p. m. Immediately after spraying the plants with the inoculated water, they were covered with bell jars or plugged lamp chimneys for 48 hours to maintain a humid atmosphere for a sufficient length of time to produce infection. Checks were carried simultaneously in all cases and given treatment in every way identical to that given inoculated plants, except that they were sprayed with sterile water instead of that laden with spores.

Series I. On Jan. 9, 1911, spores from the original culture of the *Colletotrichum* were sprayed on three (3) *Schizanthus* plants. There were two (2) control plants. All three (3) inoculated plants contracted the disease while both controls remained healthy. The period of incubation was seven days.

Series II. On Jan. 15, 1911, two (2) *Schizanthus* plants were sprayed with spores from the original culture of the fungus. After an incubation period of 8 days, both plants showed infection. The two (2) controls remained healthy.

Series III. On Jan. 23, 1911, spores from the original culture were sprayed on three (3) bean seedlings and three (3) potato plants. There were two (2) controls of each. No infection occurred and controls remained healthy.

Series IV. Jan. 28, 1911. For this set of inoculations the plants were sprayed with spores from the reisolated cultures obtained from *Schizanthus* plants showing infection as a result of inoculations made on Jan. 9, 1911. Spores were sprayed on three (3) bean seedlings, three (3) young potato plants and five (5) *Schizanthus* plants. There were two control plants of each. After an incubation period of 6 days all inoculated *Schizanthus* plants showed abundant infection. None of the bean seedlings or potato plants showed infection. All controls remained healthy.

Series V. Feb. 6, 1911. The original culture was used. Three (3) tomato seedlings and one (1) mature pepper plant which was in blossom were inoculated with spores and one (1) mature pepper plant with mycelium from the culture. There were three (3) control tomato seedlings and one (1) pepper. No infection occurred from inoculation. The controls remained healthy.

Series VI. March 20, 1911. The fungus used in this series was isolated Jan. 25, 1911, from an old infected *Schizanthus* plant growing in the Plant Breeding greenhouse. Twenty-five (25) tomato seed-

lings, five (5) bean seedlings, five (5) pepper seedlings, four (4) young potato plants, and eleven (11) *Schizanthus* plants, of two different unknown varieties both seedlings and mature, were inoculated with spores. Twenty-five (25) tomato seedlings, five (5) bean seedlings, two (2) pepper seedlings, two (2) young potato plants and five (5) *Schizanthus* plants were carried as controls. All inoculated plants remained healthy except the *Schizanthus* where 100% infection occurred. The period of incubation varied from 7 to 10 days according to the age and variety of the *Schizanthus*. Older plants did not show evidence of the disease as soon as the younger and more succulent plants. All controls remained healthy.

Series VII. April 4, 1911. The original culture was used. Fourteen (14) *Schizanthus*, ten (10) bean seedlings of the Turtle Soup and Refugee Wax varieties, five (5) tomato plants from 4 to 10 inches high, and fifteen (15) pepper seedlings with cotyledons still attached were inoculated. Three (3) *Schizanthus*, two (2) bean seedlings, two (2) tomato plants, and eight (8) pepper seedlings with cotyledons attached were used as controls. No infection occurred from the inoculations except on *Schizanthus* where 100% contracted the disease. The period of incubation was 6½ to 7 days. All controls remained healthy.

Reisolations of the fungus were made from plants artificially inoculated in each series. It is to be noted that in Series III a reisolation was used for inoculation purposes, yielding positive results. It is also to be noted that in Series VI, the culture used for the inoculations was neither the original nor a reisolation but a culture isolated Jan. 25, 1911. Attention is called to these points to emphasize, that the inoculations were not confined entirely to the use of the original culture and a single strain.

The inoculations on tomatoes, potatoes, and peppers were for the purpose of determining whether this *Colletotrichum* is not one of those which has been reported on these members of the family Solanaceae to which *Schizanthus* belongs.

Since our inoculations on *Schizanthus* were positive we feel justified in concluding that the fungus under observation is pathogenic; since our inoculations on other Solanaceous plants were negative in all cases, we conclude that the *Colletotrichum* is not one of those already reported as occurring on these hosts; and furthermore as we can find no reference in literature to a *Colletotrichum* attacking *Schizanthus*, it is to be considered an undescribed species. We therefore propose the following name for the fungus and present a technical description:

Colletotrichum schizanthi SP. NOV.

Maculis in caulibus, ramnis et petiolis depressis, irregularibus, ochraceo-brunneis; maculis in foliis orbicularibus, brunneis, 1-3 mm. in diam.; acervulis sparsis vel subgregariis, tectis dein erumpentibus; setulis numerosis, brunneis, erectis, rigidis, integris, vel uniseptatis, basi inflatis, 50-80 x 4-7μ; conidiis continuis, hyalinis, oblongis, granulatis, 12-21 x 4-7μ; basidiis hyalinis, cylindraceis, conidio aequantibus vel longioribus.

Hab. in caulibus, ramnis, petiolis et foliis vivis, *Schizanthus* sp. Ithaca, N. Y.

We are indebted to Mr. Robert Evans of the Department of Plant Breeding for providing us with plants for inoculation purposes.

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WATER CORE OF APPLE

J. B. S. NORTON

While I have seen examples of this disease at various times for several years past, my attention was especially called to it in the summer of 1908 when a student working on orchard diseases during the summer brought in samples of water core from the Experiment Station orchard. In examining sections of the diseased fruits a very large hypha of some fungus was detected in the affected tissue. Cultures of this and other specimens examined later in the year developed an Aspergillus in a number of cases, but no further evidence of a parasite could be found. During the summer of 1909, several trees of Fall Pippin and some others in the orchard of the Maryland Experiment Station were found to be badly affected by this disease and a study of the conditions affecting these trees has led me to a physiological explanation of the disease.

The affected apples are indicated by hard watery areas in the flesh, usually in the core and extending outward from it especially from the outer edge of the seed cavities. Sometimes small spots may be scattered through the flesh and there may be extensive watery areas near the surface of the fruit but not without normal flesh between them and the epidermis. In practically all cases I have noticed, the abnormal areas were in connection with vascular tissue. The seed cavities usually contained liquid and all had the hard inner membrane of the carpels cracked and covered with the hair like out-growth known as tufted carpels (the wollstreifen of Sorauer). The diseased fruit has a peculiar sweetish fermented flavor. The hard watery parts seem to contain more sugar and less acid than the normal portions, but I have made no chemical tests for these substances. A microscopic examination shows the intercellular spaces, so conspicuous in the normal apple flesh, to be filled with fluid in the diseased tissue so that the white opaque appearance of the normal flesh is lacking. No other difference was noticed except a slightly more yellow color of the cells in some samples.

Of the six Fall Pippin trees in the Maryland Experiment Station orchard, five were badly affected with water core, while one which seemed to be suffering slightly for water, owing to a diseased root system, had practically none of the water core. Apples near the top of the trees and on the sound part of branches that had been cut back by fire blight, (which had destroyed a large part of the vigorous twigs of this variety), were affected more than shaded apples or the slightly

withered ones on the lower part of slightly blighted branches. The disease was also seen on Early Harvest, Rambo and some other varieties. It seems most abundant on summer or fall apples.

The occurrence of the disease under conditions favoring excessive sap pressure or cell turgor, on vigorous growing trees, or trees with the foliage reduced by blight, and especially in late summer when the air is cold at night and the soil warm, the cracks in the carpels, the occurrence along the vascular tissue, the liquid filling the intercellular spaces, lead me to the conclusion that the trouble is due to sap forced into the seed cavities and intercellular spaces by excessive sap pressure, under conditions of reduced transpiration. The air being excluded from the inner cells by the liquid filling the intercellular spaces, anaerobic respiration may be increased and may account for the alcoholic flavor, if not lead to the decrease in acid and the sweeter taste. The flavor of the diseased fruits resembles that of frozen fruit and although I am unable to cite the publication I have recently seen the statement that the compounds formed in frozen fruits are similar to the products of alcoholic fermentation in anaerobic respiration. After discussing the subject with Dr. C. O. Appleman another explanation of the excessive turgor in the cells of the fruit has occurred to me depending on the rapid conversion of starch into sugar in the ripening processes of the fruit. Since my work on this subject is only a bi-product of other investigations I have not taken time to make any chemical tests or to try to produce the water core effect experimentally, except that Fall Pippin twigs with nearly ripe apples attached were connected with a tube of water and placed under a pressure of two atmospheres by means of a column of mercury above the water. Water was readily forced through any cuts or breaks in the twig, but no change was noticed in the fruit.

As a result of a recent search for publications on the subject I can only report the following:

Sorauer (Handbuch Pflanzenkrankheiten, **1**: 142. 1886) describes a similar or identical disease. He holds it to be non-parasitic and in the third edition of his Handbuch (1909) he cites Aderhold (Zeitschr. Pflanzenkr. **5**: 8. 1895) as maintaining similar views.

Lindau (Sorauer, Handbuch Pflanzenkr. **2**: 55. 1908) discusses the same disease and cites Prillieux (Bull. Soc. Bot. France **33**: 600. 1896) as attributing it to bacteria.

Very little occurs in American publications except bare mention of the disease as if it were well known, for example:

Arthur and Golden, Purdue Univ. Agri. Exp. Station Bull. 39: 61. 1892.

Craig, Canada Experimental Farms Report for 1896 : 172.

Selby, Ohio Agric. Exp. Station Bull. 214 : 374. 1910.

An unsigned note in Country Gentleman **68** : 1084, 1903, supposes "that the cell walls within the fruit tissue are thin and porous and that the liquids, especially water, which normally are confined chiefly in the interior of the cells break through and fill the interstices between the cells," and says also that "high cultivation or excessive rain at ripening time sometimes seems to aggravate the trouble."

(Craig and Selby (l.c.) mention core-rot following water-core conditions, and the core-rot of Baldwin which Eustace describes (N. Y. Agri. Exp. Sta. Bul. 235. 1903) as occurring during an unusually wet season may be the last stage of water-core. It seems reasonable that the abnormal respiration conditions in the water-core tissue would lead to the quicker death of such parts and a non-parasitic decay would follow.

Evans (Transvaal Dept. Agri. Technical Bull. 1. 1909) attributes bitter pit of apple in Africa to conditions somewhat similar to those here considered to be associated with water core.

Atkinson (Cornell Univ. Agri. Exp. Station, Bull. 61: 1893) has described an edema of apple twigs due to abnormal sap pressure following severe pruning and excessive cultural conditions.

Bigelow, Gore and Howard (U. S. Dept. of Agric. Bureau of Chemistry, Bull. 94. 1905) quote Lechartier and Bellamy (Compt. Rend. **69** : 356. 446. 1869) as stating that in apples standing in sealed vessels very considerable quantities of alcohol were found, but they add that "these observations have never been confirmed, neither alcohol nor yeast cells occurring in an apple whose skin is intact, however, so far as is known no work has been done since on apples held in sealed chambers." The work recorded in this bulletin showing the disappearance of starch from the core outward during the ripening of apples is very interesting and may help to explain some of the non-parasitic core decays of apples as well as the "rotten heart" of pears.

For a full discussion of anaerobic respiration in higher plants see Nabokich. Landw. Jahrb. 1909 : 50.

MARYLAND EXPERIMENT STATION

THE DIFFERENTIAL STAINING OF INTERCELLULAR MYCELIUM

ELIAS J. DURAND

For several years the writer has felt the need of a method for staining intercellular mycelium in such a way that it might be made to stand out sharply in contrast with the surrounding tissues of the host. Numerous experiments have been made from time to time, and a considerable number of combinations of staining reagents have been tried, but always with only unsatisfactory results. Inquiry has shown that other workers have met with the same difficulty, and that a differential stain for mycelium is much needed in mycological work. The mycelium of the rusts seems to be especially difficult of differentiation. Since these plants are so commonly used in class room instruction, it is all the more desirable that a simple and certain method of demonstrating the mycelial strands should be available.

Recently, when it became necessary to make a series of slides of aecia on barberry leaves for class use, the old problem again presented itself, and led to further experimentation. The results were so satisfactory that seventy-five slides were finally prepared in which the mycelium stands out in beautiful contrast with the cells of the host. The same method was then tried on *Peronospora parasitica*, in the stems of some crucifer, with equally pleasing results, the haustoria being brought out very prominently. The details of procedure are given below in the hope that others may profit by my experiences.

1. Stain the sections rather deeply with Delafield's hematoxylin; rinse in water and intensify by dipping in a glass of water to which three or four drops of ammonia have been added. The hematoxylin stains the cell walls, cytoplasm and nuclei of the host, and is of special value as a cell-wall stain.

2. Dehydrate by washing in 95% alcohol.

3. Counterstain 5-10 minutes in a ½% solution of eosin in 95% alcohol. The eosin stains the mycelium.

4. With a cloth remove the surplus stain from the slide and wash the sections thoroughly with 2-3 pipettefuls of carbol-turpentine clearer. Do not wash off the eosin with alcohol.

5. Wash in xylol and mount in balsam.

The hematoxylin is the strong stain diluted by adding three parts of distilled water to one of the stain. The eosin is the alcohol-soluble stain of Grübler. The length of time in the eosin must be determined by trial, but it is best to stain rather deeply. The clearer consists of

turpentine three parts and carbolic acid crystals (melted) two parts. This is by far the best clearer to be used after hematoxylin stains of all kinds. The secret of the whole process above outlined lies in clearing directly from the stain without washing.

In properly stained sections the mycelial threads are colored a deep pink in strong contrast with the blue of the tissues of the host. My preparations of barberry leaves are beautiful objects under the microscope. All the fungal elements are stained deep pink. The widely ramifying hyphae may be traced between the cells without the slightest difficulty, and frequently may be followed completely across the leaf from aecia to pycnia. For purposes of class demonstration they leave nothing to be desired.

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NOTES ON PERIDERMIUM CEREBRUM PECK, AND PERIDERMIUM HARKNESSII MOORE[1]

GEORGE GRANT HEDGCOCK

The following paper is a summary of the results of study and experiments in the greenhouses of the Bureau of Plant Industry at Washington, D. C., and in the forests of both eastern and western United States during the years 1908-1911. In all the experiments numerous control plants were used which remained free from disease during the entire period of investigation.

In 1908, from April 17 to May 12 the leaves of *Quercus rubra* L., *Q. lobata* Née, and *Q. densiflora echinoides* (R. Br. Campst) Sarg. were successfully inoculated with aeciospores from *Peridermium cerebrum* growing on both *Pinus virginiana* Mill., and *P. echinata* Mill. Both the uredinia and telia of *Cronartium quercuum* (Brond.) Arth. developed from inoculations with both species of pine. Teliospores from the infected oaks were successfully inoculated into wounds in the stems of *Pinus divaricata*, (Ait.) Du Mont de Cours. producing galls which were slow in developing, and did not appear conspicuous until the following year. Inoculations of teliospores into *P. strobus* L., and *P. silvestris* were similarly made without the formation of plainly developed galls.

In 1909, from May 11 to June 7, successful inoculations were made with aeciospores taken from *Peridermium cerebrum* on *Pinus virginiana* on the leaves of the following species of oaks: *Quercus alba* L., *Q. densiflora*, *Q. emoryii* Torr., *Q. gambelii* Nutt., *Q. lobata* Nee, and *Q. marilandica* Muenchh. resulting in the formation of the uredinia and telia of *Cronartium quercuum*. Successful inoculations were then made with urediniospores from *Quercus rubra* on the leaves of the following species of oaks: *Quercus emoryii*, *Q. gambelii*, *Q. lobata*, *Q. marilandica*, and *Q. rubra*. An abundant formation of both uredinia and telia followed this series of inoculations, showing that *Cronartium quercuum* spreads readily by means of the urediniospores. Successful inoculations were made with teliospores from oak leaves into wounds in the stems and twigs of *Pinus virginiana*, and *P. divaricata*, resulting in the formation of typical galls.

In 1910, from April 10 to June 1, the work of the previous year was repeated and extended, the following species of trees in addition to those previously named were successfully inoculated with both aeciospores and urediniospores: *Quercus californica* (Torr.) Coop.,

[1] Published by permission of the Secretary of Agriculture.

Q. coccinea Muenchh., *Q. phellos* L., *Q. prinus* L., *Q. texana* Buckl., *Q. velutina* Lam., *Q. undulata* Torr., *Q. michauxii* Nutt., *Q. minor* (Marsh) Sarg., *Q. virginiana* Mill., and *Castanopsis chrysophylla* (Hook) D. C. Successful inoculations were made with teliospores on the following additional species of pines: *Pinus ponderosa* Lars., *P. murrayana* "Oreg. Cm.," and *P. silvestris*, resulting in the formation of typical galls.

The galls resulting from inoculations vary in shape from globoid to oblong, or may be more or less irregular in shape. They are frequently accompanied by a formation of dwarfed clustered twigs, a sort of "witches broom" formation, especially on *Pinus ponderosa*, *P. murrayana*, and *P. virginiana*. The same witches-broom formation occurs occasionally in nature with the galls of *Peridermium cerebrum* on *Pinus virginiana* and *P. rigida* Mill., and those of *Peridermium harknessii* on *Pinus ponderosa*, *P. murrayana*, and *P. radiata* Don, in the western forests of the United States. Specimens of this form on *Pinus ponderosa*, and *P. radiata* have been collected by Dr. E. P. Meinecke of this office.

The aecial form of fruiting in *Peridermium cerebrum* is preceded for two weeks by caulicolus scattered pycnia, which exude abundantly a yellowish, sweet fluid massed with small obovate hyaline pycnospores measuring about $4\mu \times 2\mu$ in diameter.

Peridermium harknesii resembles *P. cerebrum* so closely that all that is required to prove the identity of the two species is proof that the former also has *Cronartium quercuum* for its alternate form. Inoculations have been made repeatedly this year on oaks with aeciospores from the galls of *Peridermium harknessii* on *Pinus radiata* received from California but with negative results.

All attempts to inoculate pines with *Cronartium quercuum* have failed in the absence of wounds. In nature, young Peridermium galls are often found associated with wounds made by an insect. Most galls originate on young twigs, one year old. Some originate, however, on older limbs, and even on the trunks of older trees, and may gain entrance through wounds made by birds (sapsuckers), but this hypothesis remains to be proven.

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A PECAN LEAF-BLOTCH

F. V. RAND

(WITH THREE FIGURES IN THE TEXT)

A Mycosphaerella leaf-blotch of pecans has been under study by the writer during the past year. Diseased specimens from various sections of Alabama, Georgia, Florida, South Carolina, and Ohio have been examined, field observations made in South Carolina and Georgia, and cultural studies carried out upon various media.

The disease first becomes visible on the lower surface of the leaf during midsummer or early fall in the form of dark colored blotches made up of minute, black, spherical perithecia scarcely visible individually without the aid of a lens. These perithecia are densely gregarious and occur in scattered irregular groups which often become confluent so that in many cases almost the entire lower surface of the leaf may be affected. They are at first covered by the epidermis, but finally burst through and appear more or less superficial.

Upon the mature nut shucks have been found traces of immature perithecia which may possibly be found referable to this fungus. Also multicellular conidia suggesting a Helminthosporium in several cases have been found in close connection with the immature perithecia on the leaves and may perhaps prove to be a conidial form. Single spore cultures of these conidia, however, have thus far failed to germinate and hence cultural connection has not yet been established.

There seems to be no connection whatever between this fungus and that causing the well known pecan scab, *Fusicladium effusum* Wint. The writer has studied both fungi in field and laboratory and has found no reason for connecting the two, but on the contrary, many reasons for not doing so. It is true that both fungi are sometimes found in the same orchard but, on the other hand, each often occurs where no trace of the other can be found. The Fusicladium has been obtained in pure culture from several different sources and has been carried in culture for two seasons. The pecan Mycosphaerella has been carried in culture for one season. As a result of these studies the two have been found to differ markedly in color, mycelial growth and spore formation,—in fact in nearly every cultural character observed.

It is difficult to say just how much injury is caused by this fungus. Since it comes rather late in the season, after the nuts have attained considerable size and a large part of the vegetative growth has been made, the damage is far less than that done by the pecan scab. However, any parasite which invades the inner leaf tissues and is capable

of covering a large part of the total leaf area, must interfere to a considerable extent with the physiological processes of the plant. The disease occurs to a considerable extent in the nursery, and it is here, in all probability, that the greatest damage is done.

In all the fresh material examined the writer has yet to find a single mature perithecium. In damp chamber, however, ripe asci have been readily developed on the leaves in ten days to three weeks, and these have furnished the source for the single spore cultures studied.

It was noticed that in damp chamber the perithecia often developed in the upper part of the leaf tissue and pushed through the upper epidermis at maturity. On fresh leaves gathered from the tree, however, the perithecia have always been found to occupy the lower side of the leaf.

No complete description of a species of Mycosphaerella (or Sphaerella) corresponding in characters to the pecan Mycosphaerella has been found in mycological literature. But in Saccardo an incomplete description by Thümen of a Sphaerella occurring on *Carya tomentosa* was found, which, so far as it went, corresponded with the fungus in question. A translation of this description follows:

Sphaerella convexula (Schwein.) Thüm. Mycotheca universalis n. 1149; Sphaeria convexula Schwein., Syn. No. American Fungi n. 1788; Grevillea IV. 154. Sparsely scattered over the whole leaf, buried in the host tissue, minute, roughened but shining, black, subastomous, strongly convex, not crumbling; ascigerous at the center (imperfectly observed). Hab. on leaves of *Carya tomentosa* Bethlehem and Aiken, North America (Schweinitz and Ravenel). Number 1149 Mycotheca universalis shows immature specimens entirely.

The fungus was first described by Schweinitz in his Synopsis of North American Fungi as *Sphaeria convexula*. This discription as communicated to the American Philosophical Society at Philadelphia in 1831 was left essentially unchanged by Thümen when he placed the fungus under the genus Sphaerella. Specimens of diseased leaves collected by Ravenel have been carefully examined and found to correspond with the pecan Mycosphaerella not only in general appearance and in the fact that the perithecia were immature, but in all other points observable in material not fully developed.

In view of these facts it has been decided to refer the pecan fungus to the *Sphaeria convexula* of Schweinitz (*Sphaerella convexula* of Thümen). But on account of the previous publication and present use of *Sphaerella* as the name of a genus of the Volvocaceae the writer prefers to follow Engler und Prantl in the use of the genus name *Mycosphaerella*. The following revised and completed description is therefore submitted:

Mycosphaerella convexula (Schwein.) N. COMB.

Sphaerella convexula (Schwein.) Thüm., Sacc. I. 494. *Sphaeria convexula* Schwein., Syn. No. Amer. Fungi no. 1788. Mycelium septate, olive-brown, intercellular (partly white or pink in culture); perithecia mostly hypophyllous, densely gregarious, in irregular isolated groups or scattered more or less over the entire leaf, at first buried in the host tissue, finally bursting through the epidermis, minute, 100-200μ in diameter (often somewhat larger in culture), somewhat roughened superficially, shining black, sphaerical or nearly

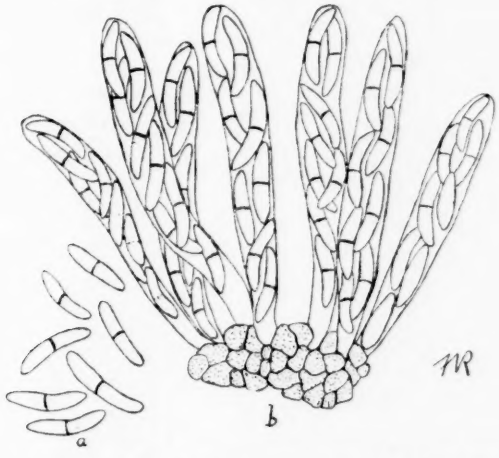

FIG. 1. Ascus cluster, free ascospores and hyphae developed on corn meal agar. x 540.

so, at maturity papillate and perforated at the apex, not crumbling; paraphyses lacking; asci fasciculate, cylindrical-clavate, 54-100 x 9-11μ in diameter, eight-spored; ascospores allantoid, sometimes oblong-fusiform, one-septate, often slightly constricted at the septum, the two cells similar, hyaline, 13-27 x 3.5-5.5μ in diameter. Hab. living leaves of Carya spp. in the southern United States. Perithecia coming to maturity on decaying leaves.

This fungus, obtained in single spore cultures as stated above, has been grown in the laboratory for several months upon a number of different culture media. A partial summary of the results of this study follows:

On beef agar growth becomes visible in one to two days after inoculation as a colorless, roundish, submerged colony, which in five to seven days nearly or quite covers the slant. In the meantime one to several groups of black perithecia have begun to form,—these groups

from mere pin points in size up to 0.5 mm. or more in diameter. The growth is still entirely submerged and the surface of the slant presents a moist, glistening appearance. At ten days to two weeks the perithecial clusters have increased somewhat in size, and a small amount of colorless or dirty-white aerial mycelium makes its appearance at the upper edge of the slant. In old cultures (one or more months old) the agar becomes very dark colored, especially in the upper part of the slant. The surface presents a black, glistening appearance, and is very sparsely covered with a dirty-white aerial mycelium. A considerable number of black perithecial clusters are present but as yet no asci have been found in beef agar cultures. The subicle and immature perithecia are seen to consist of variously swollen cells and intricately anastomosing hyphae varying in color from hyaline to olive-brown, and in the perithecia uniting to form a false parenchyma. This appearance of the mycelium is similar with all the culture media studied.

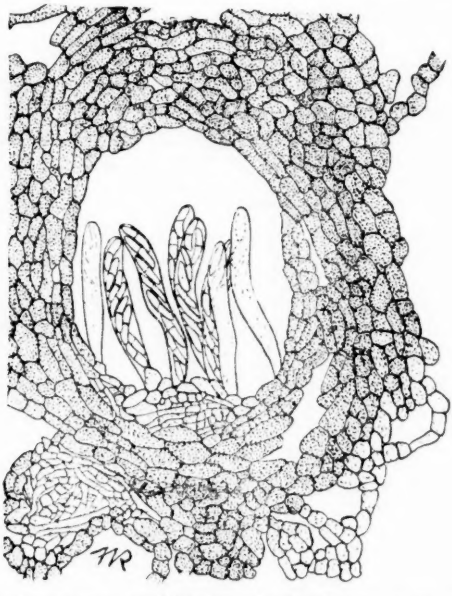

FIG. 2. Section of a mature perithecium developed on corn meal agar. x 260.

On cooked corn meal growth becomes visible in two to three days after inoculation as a roundish colony 5-15 mm. in diameter, with cottony, white to pale pink, but very sparse aerial mycelium in which are scattered a considerable number of dark olive-brown dots. These dots are found to consist of numerous variously interwoven hyphae with swollen cells which seem to be in process of uniting to form a

pseudoparenchyma. In a week to ten days the colony attains a diameter of 60 or more mm. and in the older parts presents a dense aggregation of round, black perithecial clusters borne in a rather sparse pinkish-white subicle. All gradations occur between these mature perithecial clusters at the center, and the small, roundish masses of olive-brown anastomosing hyphae near the margin of the colony. Asci and ascospores are readily developed on this medium in ten days to three week after inoculation.

On corn meal agar growth becomes visible in one to two days as a roundish colony one or more mm. in diameter, and with entire margin. The mycelium is colorless and entirely submerged. After about a week the colony covers the slant and a few scattered perithecial clusters make their appearance, projecting somewhat above the surface of the medium. The vegetative mycelium, however, remains submerged except sometimes for a small amount of cottony white aerial mycelium

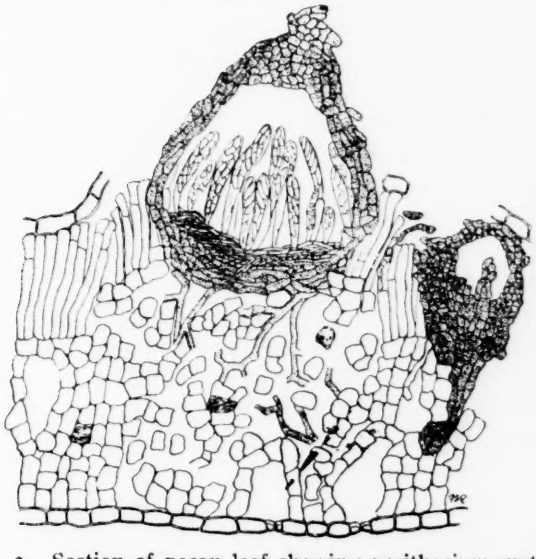

FIG. 3. Section of pecan leaf showing perithecium matured in damp chamber. x 180.

at the upper part of the slant. The colony presents a slight radiating appearance from the point of inoculation outward and the surface is smooth and glistening. Old colonies present essentially the same appearance as those of one or two weeks, except that the perithecial clusters are somewhat larger and more numerous and sometimes a tinge of olive green appears in the vegetative mycelium. Only scattered groups of perithecia appear on corn meal agar but asci readily develop and mature.

On oxalic acid agar growth is somewhat more tardy, colonies rarely becoming visible before the second or third day after inoculation. The mycelium is mostly at the surface of the medium at first, and possesses no color distinct from the latter. The colonies are nearly round, and with entire margin. After four to seven days a dense ring of cottony, pinkish aerial mycelium makes its appearance around the center and keeps pace with the growth of the colony, but always one to several mm. within the margin. In old cultures the red color of the medium has disappeared, and sometimes one to several perithecial clusters are present, generally near the center of the colony. No mature perithecia have as yet been found on this medium.

On cooked potato cylinders growth first becomes evident by a light-brown discoloration of the cylinder immediately around the point of inoculation, and generally a tuft of whitish aerial mycelium appears within 24 hours at the center of the discolored area. The latter increases rapidly in extent until in a week's time nearly the whole cylinder has turned a pale, chocolate-brown. The cottony subicle assumes a very pale pink color, and gradually increases in extent but always somewhat behind the margin of the discolored area. In old cultures this pinkish subicle nearly covers the surface of the cylinder and bears numerous clusters of black perithecia, many of which contain mature asci.

In addition to the cultural studies at laboratory temperature several series of cultures were run in a nine compartment incubator with temperature ranging from one to eighteen degrees Centigrade. As a result of these studies it was found that no growth would take place below six degrees, either with freshly inoculated cultures or with cultures in which growth had already started before placing in the incubator. At seven to eight degrees growth was extremely slow, but gradually increased with raise of temperature until the maximum, for the temperatures tested, was reached at eighteen degrees. At the latter temperature the colonies covered the slants after seven to ten days incubation.

A further study of this fungus is being carried out in laboratory, greenhouse, and field; and experiments in control methods are under way.

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